

Heavy Metal Pollution and Antioxidant Defense Responses in Freshwater Fishes

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ABSTRACT

Heavy metal contamination has become a pressing environmental concern in freshwater ecosystems, particularly in river systems impacted by industrial discharge, agricultural runoff, and domestic effluents. In rivers of Punjab Pakistan, elevated concentrations of chromium, lead, cadmium, nickel, and manganese have been reported in water and fish tissues, especially in commercially important species such as *Labeo rohita* and other cyprinids. These non-degradable metals bioaccumulate in fish through direct absorption and trophic transfer, disrupting physiological and biochemical processes. A principal mechanism of toxicity involves excessive production of reactive oxygen species (ROS), leading to oxidative stress when antioxidant defenses are overwhelmed. ROS-induced lipid peroxidation, commonly assessed via malondialdehyde (MDA) levels, damages cellular membranes, proteins, and DNA. Fish counteract oxidative stress through integrated antioxidant systems, including enzymatic components (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathione-S-transferase) and non-enzymatic molecules such as glutathione and vitamins C and A. However, responses are dose-, tissue-, and species-specific, often showing adaptive enzyme induction at low metal concentrations and system failure at higher exposures. Biomarker-based assessments, including enzyme activity assays and metal accumulation analyses, provide valuable tools for ecological risk evaluation. Monitoring antioxidant responses in sentinel fish species offers an effective early warning framework for pollution management and conservation strategies in contaminated freshwater basins.

Keywords: Antioxidants, defense, fisheries, heavy metals, Rivers

To cite this article: Mehmood R, M Afzaal, F Ahmad, A Yousaf, M Iram & U Liaqat. Heavy Metal Pollution and Antioxidant Defense Responses in Freshwater Fishes. *Biological Times*. 2026. September 5(9): 19-21.

Introduction

Heavy metal pollution in freshwater ecosystems has emerged as a critical environmental issue worldwide, stemming largely from industrial effluents, agricultural runoff, and domestic waste discharge. These non-degradable contaminants accumulate in aquatic environments and are readily taken up by resident biota, particularly fish, through water and sediment exposure and trophic transfer. Once internalized, heavy metals such as chromium (Cr), lead (Pb), cadmium (Cd), nickel (Ni), and manganese (Mn) can bioaccumulate in fish tissues, leading to disruptions in physiological and biochemical processes (Ensibi et al., 2012; Jezierska & Witeska, 2006).

Freshwater fishes are especially vulnerable to heavy metal-induced toxicity due to their intimate and continuous interaction with polluted waters. Heavy metal exposure can trigger excessive production of reactive oxygen species (ROS), unstable molecules that interact destructively with cellular components including lipids, proteins, and nucleic acids. When ROS generation surpasses the organism's inherent antioxidant capacity, oxidative stress ensues—ultimately compromising cellular integrity and organismal health (Lushchak, 2011; Stohs & Bagchi, 1995).

To mitigate oxidative damage, fish possess a suite of antioxidant defense mechanisms comprising enzymatic and non-enzymatic components. Key enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) act synergistically to neutralize ROS, whereas non-enzymatic molecules like reduced glutathione (GSH) and metal-binding proteins such as metallothioneins play crucial roles in detoxification and metal sequestration (Halliwell & Gutteridge, 2015).

In the Rivers, major freshwater systems, anthropogenic activities including industrial discharge and agricultural runoff have been reported to elevate heavy metal concentrations in both water and fish tissues. Studies have documented significant accumulation of heavy metals in commercially important species such as *Labeo rohita* and other cyprinids, highlighting ongoing contamination threats to aquatic life and potentially human consumers (Ali & Khan, 2019; Qadir & Malik, 2011).

Understanding the antioxidant defense responses of freshwater fishes to heavy metal stress is therefore essential for assessing sub-lethal impacts of pollution, elucidating adaptive physiological mechanisms, and evaluating the use of antioxidant biomarkers as indicators of environmental health. This knowledge forms a fundamental basis for ecological risk assessment and the development of effective conservation and pollution management strategies in the River basin.

2.1. Oxidative Stress Mechanisms in Fish

Reactive Oxygen Species Generation: Aerobic organisms including fish continuously generate ROS such as superoxide anion radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\cdot OH$) as byproducts of

oxidative metabolism, which can initiate lipid peroxidation in tissues. These ROS target polyunsaturated fatty acids in lipoproteins, essential components of cell membranes, with disruption of lipid membrane structural properties potentially causing serious cellular dysfunction ((Ensibi et al., 2012).

Lipid Peroxidation Process: The hydroxyl radicals initiate lipid peroxidation which generates reactive electrophiles such as epoxides and aldehydes, with malondialdehyde (MDA) serving as a primary secondary lipid oxidation product that functions as a reliable biochemical indicator of oxidative damage ((Bayir et al., 2011; Ensibi et al., 2012). Fish tissues contain large quantities of unsaturated lipids (PUFAs), making them particularly vulnerable to oxidative stress since these lipids represent major targets for ROS (Bayir et al., 2011).

Cellular Damage Pathways: Oxidative stress occurs when ROS generation exceeds removal capacity, leading to damage of DNA, proteins, steroid components, and unsaturated lipids in cell membranes. The typical reaction during ROS-induced damage involves peroxidation of unsaturated fatty acids, with pollutant-induced lipid peroxidation (LPO) observed across multiple fish species, though response levels vary by species (Ahmad et al., 2000) (Figure 1).

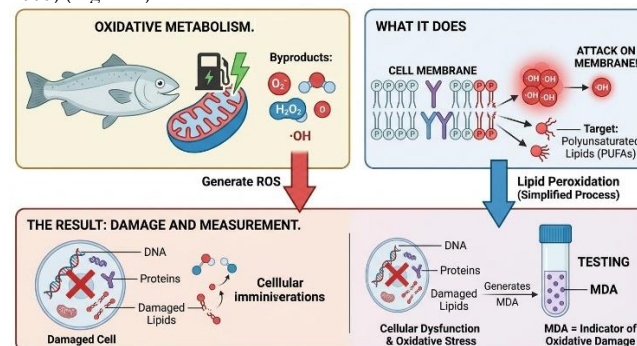


Figure 1: Pathways of Reactive oxygen species (ROS) induced lipid peroxidation in fish tissues.

2.2. Antioxidant Defense Systems in Fish

Enzymatic Defense Mechanisms: Fish possess an antioxidant defense system similar to other vertebrates utilizing both enzymatic and non-enzymatic mechanisms. The most important antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-S-transferases (GSTs) (Bayir et al., 2011; Ensibi et al., 2012). SOD catalyzes the dismutation of superoxide radicals to molecular oxygen and hydrogen peroxide, CAT

degrades hydrogen peroxide into water and oxygen, while GPx reduces hydrogen and organic peroxides using glutathione as an electron donor (Castro et al., 2012).

Non-Enzymatic Defense Components: Low molecular weight antioxidants such as glutathione (GSH), ascorbate (vitamin C), and vitamin A contribute to quenching oxyradicals (Al-Harbi et al., 2014; Sharbidre et al., 2011). Glutathione serves as an important antioxidant readily oxidized by ROS to oxidized glutathione, but can also bind metals that might induce oxidative stress (Firat & Kargin, 2010). Vitamin C is a strong antioxidant capable of scavenging ROS and regenerating other antioxidant molecules such as α -tocopherol (Al-Harbi et al., 2014).

Integrated Defense Strategy: Fish maintain complex systems of multiple antioxidant types that work in concert to prevent oxidation of other molecules and protect organisms from oxidative damage (Liu et al., 2010). The antioxidant status in fish can be manipulated through feed to cover basic vitamin requirements, prevent degenerative diseases, improve immunity, and enhance resistance to infectious diseases (Blazer, 1992).

2.3. Heavy Metal-Induced Oxidative Stress

Metal-Specific Mechanisms: Heavy metals such as lead (Pb), cadmium (Cd), copper (Cu), mercury (Hg), silver (Ag), and cobalt (Co) have been shown to be extremely toxic when binding to fish gills. These metals exert toxicity by generating free radicals including hydroxyl radical ($\cdot\text{OH}$), peroxy-radical ($\text{RO}_2\cdot$), and superoxide ($\text{O}_2\cdot^-$), as well as non-radical ROS such as hydrogen peroxide (H_2O_2) (Siwela et al., 2009).

Cellular Binding Effects: Mercury can alter enzyme activity by binding to functional groups or displacing associated metals, with its primary mechanism of cellular toxicity involving binding to sulfhydryl ($-\text{SH}$) groups by forming disulfide bridges in proteins, resulting in conformational changes that prevent normal protein functions (Guilherme et al., 2008). Chromium exposure results in oxidative stress through production of ROS including superoxide anion ($\text{O}_2\cdot^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical (Li et al., 2013).

Dose-Response Relationships: The antioxidant response to heavy metals follows a biphasic pattern where low concentrations may induce protective enzyme upregulation while higher concentrations overwhelm defense mechanisms (Dautremepuits et al., 2002; Tort et al., 2005). For example, in freshwater fish exposed to sublethal concentrations of cadmium chloride, tissue antioxidant enzymes function to protect the organism from toxicity before metallothionein synthesis occurs (Tort et al., 2005).

3. Research Methods and techniques to measure the stresses

3.1. Biomarker Assessment Techniques

Enzyme Activity Measurements: Standard methodologies include spectrophotometric assays for measuring superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione S-transferase (GST) activities in various fish tissues (Ahmad et al., 2000; Siwela et al., 2009). These enzymes are typically measured in liver, gills, and kidney due to their critical roles in detoxification and high exposure to contaminants (Siwela et al., 2009).

Lipid Peroxidation Quantification: Malondialdehyde (MDA) levels are commonly measured using thiobarbituric acid reactive substances (TBARS) assay as an indicator of lipid peroxidation and oxidative damage (Guilherme et al., 2008). This parameter provides a reliable measure of membrane damage resulting from ROS exposure (Bayir et al., 2011).

Metal Accumulation Analysis: Tissue metal concentrations are typically determined using atomic absorption spectrophotometry or inductively coupled plasma mass spectrometry to establish correlations between metal accumulation and antioxidant responses. The liver is frequently targeted due to its role in detoxification and importance to individual fish health (Guilherme et al., 2008).

3.2. Experimental Design Approaches

Exposure Protocols: Studies typically employ controlled laboratory exposures using sublethal concentrations of specific metals for defined durations (acute: hours to days; subchronic: weeks; chronic: months) (Bayir et al., 2011; Ensibi et al., 2012). Field studies collect fish from contaminated sites and compare them with reference populations from relatively clean areas (Tort et al., 2005).

Tissue-Specific Analysis: Research consistently demonstrates tissue-specific responses to heavy metal exposure, with liver generally showing greater resistance to oxidative damage compared to gills and kidney. This differential response necessitates multi-tissue analysis to fully characterize antioxidant responses (Van der Oost et al., 2003).

Time-Dependent Assessments: Longitudinal studies track changes in antioxidant parameters over time to distinguish between adaptive responses and progressive damage (Ali & Khan, 2019). For example, metallothionein induction in *Channa punctatus* liver occurred after 60 days of paper mill effluent exposure, while catalase activity showed an initial decrease followed by later increase.

4. Case Analysis

4.1. Metal-Specific Response Patterns

Cadmium and Copper Effects: In carp (*Cyprinus carpio* L.) exposed to copper, antioxidant responses were modulated by both the metal concentration and parasitic infection status, demonstrating the complex interactions between environmental stressors and biological factors. Similarly, freshwater fish exposed to sublethal cadmium concentrations showed tissue antioxidant enzymes functioning as the first line of defense before metallothionein synthesis occurs (Canli et al., 1998).

Mercury Accumulation Dynamics: In *Liza aurata* exposed to environmental mercury, the liver was targeted due to its detoxification role, while mercury residue in muscle represented the major route of human exposure, primarily as methylmercury (MeHg). Mercury inhibited ethoxyresorufin-O-deethylase (EROD) activity, a functional assessment of CYP1A, even at low concentrations, potentially reducing fish ability to metabolize and excrete xenobiotics (Mieiro et al., 2011).

Chromium-Induced Responses: Pengeze crucian carp (*Carassius auratus* var. Pengeze) exposed to hexavalent chromium showed modulation of antioxidant enzymes including CAT, GPx1, and Cu/Zn-SOD, with molecular cloning revealing specific gene expression changes in response to metal stress (Li et al., 2013). This demonstrates how heavy metals can trigger both enzymatic activity changes and underlying genetic responses.

4.2. Tissue-Specific Vulnerability

Liver vs. Gill Responses: Studies consistently show that liver exhibits greater resistance to oxidative damage compared to gills and kidney. In *Channa punctatus* exposed to paper mill effluent, the liver showed a time-dependent increase in glutathione level and activities of glutathione peroxidase and glutathione S-transferase, while catalase activity initially decreased before increasing after 60 days (Ahmad et al., 2000).

Erythrocyte Sensitivity: Erythrocytes are particularly vulnerable to oxidative stress due to their role in oxygen transport, with approximately 3% of oxyhemoglobin undergoing autooxidation and generating superoxide radicals (Livingstone, 2001). In *Oreochromis niloticus* exposed to zinc and cadmium, erythrocyte antioxidant systems showed significant alterations, with glutathione concentrations changing in a time- and dose-dependent manner (Siwela et al., 2009).

Species-Specific Variations: Different fish species exhibit varying susceptibility to oxidative stress based on their evolutionary adaptations. Elasmobranchs show higher lipid peroxidation levels than marine teleosts, which have higher levels than freshwater teleosts, with these differences attributed mainly to variations in antioxidant mechanisms (Ahmad et al., 2000) (Figure 2).

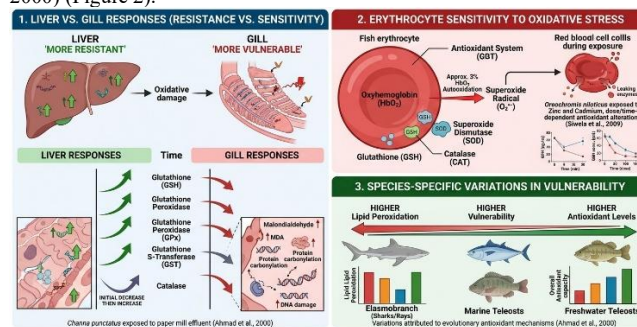


Figure 2: Tissue specific vulnerability to antioxidant stress in fish

4.3. Adaptive vs. Damaging Responses

Protective Enzyme Induction: When freshwater fish *Channa punctatus* preexposed to low copper concentrations were subsequently exposed to endosulfan, protection against oxidative damage was observed in the liver, demonstrating cross-protection through antioxidant induction. Similarly, catfish exposed to low mercury levels showed increased antioxidant enzyme activities, providing additional protection against mercury-induced oxidative damage (Hermes-Lima & Zenteno-Savin, 2002).

System Overload Indicators: The reduction in antioxidant enzyme activity at higher metal concentrations suggests that defense mechanisms become overwhelmed by increased ROS production induced by heavy metal stress. This transition from adaptive response to system failure represents a critical threshold in toxicological assessment (Ahmad et al., 2000).

Lipid Peroxidation as Damage Marker: Malondialdehyde levels consistently increase with metal exposure intensity and duration, serving as a reliable indicator of membrane damage (Bayir et al., 2011; Ensibi et al., 2012). In zebrafish exposed to atrazine, oxidized lipid levels increased at higher concentrations (30 and 90 $\mu\text{g L}^{-1}$), demonstrating the dose-dependent relationship between contaminant exposure and oxidative damage (Hermes-Lima & Zenteno-Savin, 2002).

5.0. Recommendations

Integrated Monitoring Programs: Environmental agencies should implement integrated biomonitoring programs that measure both metal accumulation and antioxidant responses (particularly MDA levels and key enzyme activities) in multiple fish tissues to assess river health. This approach provides early warning of pollution impacts before significant population declines occur.

Species Selection Criteria: Monitoring programs should prioritize fish species with known sensitivity to oxidative stress and well-characterized antioxidant responses, such as common carp (*Cyprinus carpio*), tilapia (*Oreochromis spp.*), and catfish (*Channa punctatus*). These species serve as effective sentinels for aquatic ecosystem health.

Pollution Mitigation Strategies: Efforts to reduce heavy metal inputs into river systems like the Chenab should focus on industrial discharge controls, improved agricultural practices to minimize metal runoff, and restoration of riparian vegetation to enhance natural filtration. Additionally, research into phytoremediation and other bioremediation approaches shows promise for reducing metal concentrations in contaminated waters.

Research Priorities: Future research should prioritize investigating the combined effects of multiple metals commonly found together in river systems, developing species-specific biomarker thresholds for different metals, and establishing the relationship between antioxidant responses and long-term fish population health. Particular attention should be given to understanding how climate change factors like rising water temperatures may exacerbate metal toxicity through increased oxidative stress.

The antioxidant defense responses in freshwater fishes exposed to heavy metal pollution represent a sophisticated biological early warning system that, when properly monitored and interpreted, can provide critical insights into river ecosystem health. By leveraging these natural defense mechanisms as biomarkers, environmental managers can implement more effective pollution control strategies to protect aquatic biodiversity and human health in river systems like the river Chenab.

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